CHAPTER 1

INTRODUCTION

1.1 Background of Study

In 2016, New Straits Times reported that diabetes is one of the top 10 deadliest diseases in Malaysia. Statistical data from National Health and Morbidity Survey 2015 revealed that the number of diabetics in Malaysia have increased to 17.5% from 15.2% in 2011 (Bernama, 2016). One of the major factors that contribute to diabetes is obesity. The British medical journal, The Lancet, show that 49% of women and 44% of men in Malaysia were found to be obese (Idris, 2016). Obesity is closely related with insulin in human body. The main function of insulin in the human body is to allow the sugar present in the blood to enter the muscle and tissue cell. When the fat in the body is increased, the muscle and tissue cells become resistance to insulin, leading to high blood sugar level in blood (hyperglycemia) and finally lead to diabetes (Czech, 2017). However, in a modern lifestyle era, people are getting concerned about their health and try to avoid taking too much sugar and high-calories food in their daily diet. This result in the high demand for stevia sweeteners in the foods market because it has no calories and not harmful as other chemical sweeteners like Aspartame and Saccharin.

Stevia rebaudiana (Bertoni) or commonly known as stevia or “sugar leaf” is a small perennial herb belonging to the sunflower family (Asteraceae). It was discovered by indigenous people in South America who used the stevia leaves to sweeten beverages and chew for the sweet taste. The compound responsible for the sweet taste in the stevia leaves is steviol glycosides. This includes stevioside, Reb A, Reb B, Reb C, Reb D, Reb E and Reb F, steviolbioside and dulcoside A (Anvari, 2016). Stevioside tastes about 300 times while reb A tastes about 400 times sweeter than 0.4% sucrose solution. The best part of stevia extract is Reb A because it has no aftertaste but stevioside has a bitter aftertaste.

Basically, there are two steps involved in the isolation of Reb A. Firstly, dried ground stevia leaves were extracted using various techniques such as solvent extraction method, supercritical fluid extraction and enzymatic extraction. Then, the stevia extract undergoes a purification process to remove color and other impurities to produce high purity of stevia. There are a lot of studies on purification method such as membrane filtration, column chromatography, preparative HPLC, liquid-liquid extraction (LLE) and adsorption using activated charcoal. The yield of Reb A was determined using analytical HPLC.

1.2 Problem Statement

Stevioside, Reb A and Reb C are the major constituents of steviol glycosides and they are responsible for the sweet taste of stevia. Among them, Reb A has the highest sweetness and high quality of taste. Nevertheless, it is very difficult to isolate Reb A from stevioside and Reb C because the chemical structures of stevioside, Reb A, and Reb C are very similar. There are a lot of purification methods used to isolate Reb A from crude stevia extract, for examples Ultrafiltration (UF) and Nanofiltration (NF) membrane, column chromatography, preparative HPLC, and crystallization technique. However, these methods might take a longer processing time, low yields, complicated process and difficult to scale up. Due to several limitations in this experiment such as availability of apparatus and equipment, time and cost, LLE method and adsorption method have been selected in this present study. The yield of Reb A was compared to identify the best method that will provide the highest extraction yield and purity of Reb A.

1.3 Research Objectives

General objective

The objective of this study is to identify the best purification method that will produce high yield and purity of Reb A from crude stevia extract.

Specific objectives

i. To determine the yield and purity of Reb A using different ratio of diethyl ether and crude stevia extract in LLE method.
ii. To study the effect of number of purification towards the yield of Reb A in LLE method.
iii. To determine the yield and purity of Reb A using different percentages of activated charcoal in adsorption method.
iv. To compare the yield of Reb A between different number of extraction in adsorption method.

1.4 Scope of the Study

In order to achieve research objectives, the following scopes have been identified:

i. To determine the effects of different ratio of diethyl ether (ml) and crude stevia extract (ml) on the yield and purity of Reb A in LLE. The ratios are 1:2, 2:2, 3:2, 4:2 and 5:2. The yield of Reb A was analysed using analytical HPLC.
ii. To study the effects of different percentages of activated charcoal (5, 10, 15, 20 and 25%) on the yield and purity of Reb A in adsorption method. The concentration of Reb A was determined by analytical HPLC.
iii. To evaluate the effect of extraction stages (stage I, II and III) on the yield of Reb A in LLE and adsorption method. The quantification of Reb A was analysed using analytical HPLC
iv. The yield of Reb A in both purification techniques were compared to determine the best method which provided high yield and purity of Reb A.